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Evaluation of potassium peroxydisulfate (MPS) efficacy against SARS-CoV-2 virus using RT-qPCR-based method

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spreads mostly through respiratory droplets, and the inhalation of these droplets is the main route of transmission leading to the fatal coronavirus disease 2019 (COVID-19) (Wang et al., 2020). Contact with contaminated fomites has been proposed as another possible route of viral transmission. Recently, the survival of SARS-CoV-2 on different surfaces has been assessed. The virus can remain viable for at least 3 hours in the air post-aerosolization, for 24 hours on cardboard, and for up to 2–3 days on domestic materials like plastic and stainless steel (van Doremalen et al., 2020). In addition, environmental contamination with SARS-CoV-2 has been identified in COVID-19 patients rooms before cleaning (Ong et al., 2020), including the intensive care unit, isolation ward, computer desktop, and even doorknobs (Ye et al., 2020). Hence, proper disinfection of the environment and surfaces that could have become contaminated with SARS-CoV-2 is necessary to reduce the risk of viral transmission.

Regarding the World Health Organization recommendation, environmental cleaning should be performed appropriately using suitable disinfectants. The US Centers for Disease Control and Prevention (CDC) have recommended disinfectants against SARS-CoV-2 (<https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/cleaning-disinfection.html>). Several disinfectants and disinfection methods have also been reviewed for their potential use for environmental decontamination during the SARS-CoV-2 pandemic (Cimolai, 2020). These include alcohol, hydro-

gen peroxide (H₂O₂), quaternary ammonium compounds, and sodium hypochlorite (NaClO). Recently, alcohol-based disinfectants (ethanol, isopropyl alcohol), H₂O₂, and NaClO have been shown to significantly reduce the viral contamination on surfaces within a 1-minute exposure time (Kampf et al., 2020). However, there are limitations to the use of these disinfectants in various circumstances; for example, NaClO and H₂O₂ are known to be corrosive agents to direct skin contact, and the disinfectant activity of alcohol falls off rapidly if the ratio is wrong or if the solution absorbs excess water.

Potassium peroxydisulfate (also known as potassium monopersulfate, MPS) is a broad-spectrum disinfectant that acts on bacterial and viral protein capsids by oxidation, thereby releasing and inactivating the nucleic acids of viruses. The bactericidal and virucidal efficacies of this salt are dependent on the concentration, contact time, and organic material conditions (Kampf et al., 2020). Due to its safety profile, MPS is widely used for multipurpose virucidal disinfection at certain concentrations (Sonthipet et al., 2018), for example in the livestock industry to disinfect animal shelters, in meat production facilities, and in swimming pools (Sykes and Weese, 2014). Moreover, MPS-based products have been shown to inactivate the severe acute respiratory syndrome coronavirus (SARS-CoV) like NaClO (Lai et al., 2005). Although MPS powder is corrosive and causes serious skin and ocular burns from direct contact, MPS solution is non-irritating and safe for animals and humans (Sonthipet et al., 2018; Sykes and Weese, 2014). Additionally, the MPS-based products that have been tested have not been classified as harmful or toxic according to the standard European process for the classification and labelling of chemical preparations. Therefore, this solution could potentially be an alternative against SARS-CoV-2 during the

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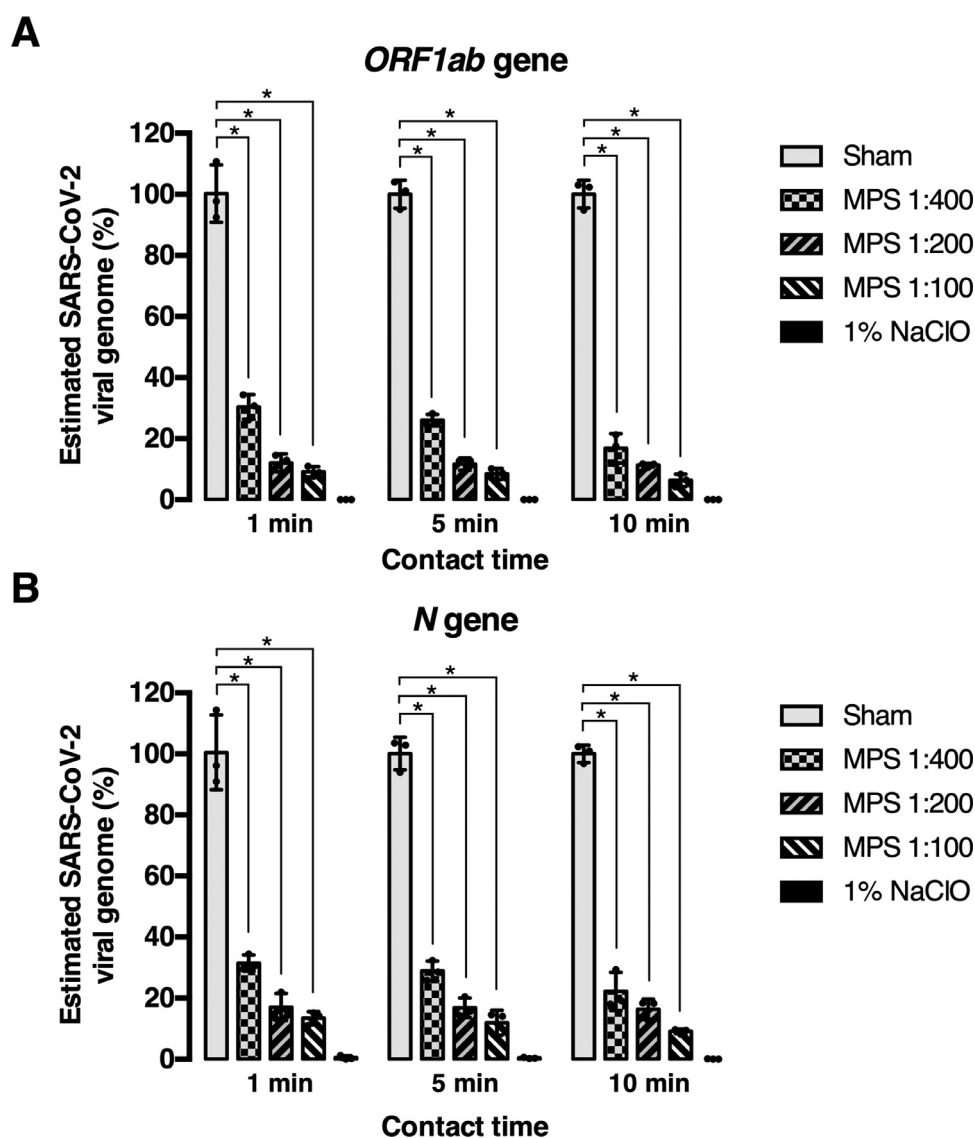


Figure 1. Effect of disinfectants on SARS-CoV-2 genomic RNA. The SARS-CoV-2 viruses were incubated with disinfectants (MPS and NaClO) at various concentrations and with various contact times. The viral RNA copies were detected using an RT-qPCR specific to the SARS-CoV-2 genomic RNA sequence by targeting the *ORF1ab* gene (A) and *N* gene (B). The average cycle threshold (Ct) value of the sham control for the *ORF1ab* gene and *N* gene at 1 minute was 30.08 ± 0.1332 and 28.45 ± 0.1721 (mean \pm standard deviation), respectively. The recommended cut-off Ct based on the manufacturer's instructions (CerTest Biotech, Spain) for the clinical diagnosis of SARS-CoV-2 infection is <38 cycles for both genes. The relative reduction (as a percentage, %) of the SARS-CoV-2 viral copies was calculated based on the Ct numbers detected for 'sham' and 'after treatment with disinfectant' in three independent experiments. The data are presented as the mean \pm standard deviation, $n = 3$. The asterisk (*) indicates statistical significance by 2-way ANOVA with Dunnett's multiple comparison test; $P < 0.01$.

MPS = potassium peroxymonosulfate; NaClO = sodium hypochlorite; ORF1ab = open reading frame 1; N = nucleoprotein.

ongoing outbreak. So far, there has been no direct evidence of its effect on this virus.

To provide the first evidence that MPS is effective against SARS-CoV-2, we performed an in vitro study using virus derived from Thai COVID-19 patients. Previous data indicated that disinfectants such as H_2O_2 and/or NaClO may disintegrate and reduce the amount of intact viral particles as determined using an RT-qPCR technique, and this was correlated with a reduction in their infectivity dose (Bowman et al., 2015). Therefore, an RT-qPCR that specifically identifies regions of SARS-CoV-2 was used to detect the relative number of viral copies using the cycle threshold (Ct) changes with and without disinfectants. At room temperature, 15 μ l of known SARS-CoV-2-positive samples in viral transport medium were first placed into the sterile plastic wells and then treated with 15 μ l of different MPS dilutions (1:100, 1:200, and 1:400 w/v; Oxipro, Thailand) for various contact times (1, 5,

and 10 minutes). We used 15 μ l of 1% sodium hypochlorite as a reference disinfectant and sterile water as a negative (sham) control. Then individual samples from each well were subjected to viral genomic RNA extraction using MagDEA Dx S.V. in-tip magnetic bead extraction with an automated magLEAD 12gC machine (Precision System Science, Japan). The CE-IVD-marked VIASURE SARS-CoV-2 Real-time PCR Detection Kit (CerTest Biotech, Spain) targeting the *ORF1ab* and *N* genes were applied to detect intact SARS-CoV-2 genomic RNA. The amplification and fluorescence signal was detected using a CFX96 Real-time PCR and C1000 thermal cycler machine (Bio-Rad, UK).

Experiments were performed in triplicate (Figure 1). It was found that all three MPS dilutions (1:100, 1:200, 1:400) significantly reduced the detection of SARS-CoV-2 by 93.7%, 88.7%, and 83.2%, respectively, based on the *ORF1ab* gene, when compared to the negative control (Figure 1A). Meanwhile, 1% NaClO induced

a >99.9% loss in SARS-CoV-2 detection. Similar results were observed for *N* gene amplification (Figure 1B). The internal controls of all RT-qPCR reactions were not significantly different, implying that the reduction in detected intact viral genome was not a result of PCR inhibition of the disinfectants ($C_t = 20.37 \pm 0.150$, 20.36 ± 0.047 , and 20.38 ± 0.026 for Sham, NaClO, and 1:100 MPS, respectively at 1-min exposure time; mean \pm standard deviation). The highest clearance rate was unsurprisingly found at 1:100 concentration, and this concentration is unlikely to affect human health based on the manufacturing information. Moreover, a longer exposure time to MPS did not increase this efficacy; the results at 1 minute were not different from those at 5 and 10 minutes. Even though the RT-qPCR results might not directly determine viral viability, based on the nature of this RNA virus, marked viral genome disintegration would tremendously affect its infectivity.

In summary, the results of this study provide the first evidence that MPS has a disinfection efficacy against SARS-CoV-2 and could be used as an environmental decontaminant in the household, hospital facilities, and food industry with an acceptable safety profile.

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Ethical approval

This work was approved by the local ethics committee at the center.

Conflict of interest

None declared.

Author contributions

V.V., A.T., and A.A. conceived of the presented idea. W.T. and V.V. designed the experimental setup. W.T. performed the experimental data collection and analysis. All authors discussed the results and contributed to the final manuscript.

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